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Lymphocyte Responses to Human Cytomegalovirus in Different Groups of Patients in Britain and in Adults From West Africa and the Middle East

K.A. Omisakin, F. Davidson, D.H. Bevan, W. Al Nakib, K.S. Abdel Wahab, and J.C. Booth

Departments of Medical Microbiology (K.A.O., J.C.B.), Genitourinary Medicine (F.D.), and Haematology (D.H.B.), St. George's Hospital Medical School, University of London, United Kingdom; Department of Microbiology, University of Kuwait, Kuwait (W.A.N.); Department of Medical Microbiology, Al Azhar University Medical School, Cairo, Egypt (K.S.A.W.)

Antibody prevalence and lymphocyte proliferation responses to cytomegalovirus (CMV) and herpes simplex virus (HSV) were compared in several different groups of patients: genitourinary medical (GUM) patients, hemophiliacs, men with clinical acquired immunodeficiency syndrome (AIDS) and cases of primary CMV mononucleosis, and also in adults in the general population (control subjects) comprising separate groups native to Britain, West Africa, and the Middle East. Among the British control subjects who were positive for CMV IgG, all were also positive against CMV antigen by the lymphocyte transformation test (LTT). However, among those who were CMV IgG-positive in the various groups of patients, 20–86.9% gave positive responses to CMV antigen by the LTT; moreover, 75.7% and 55.5% of the CMV IgG-positive healthy control subjects from West Africa and the Middle East, respectively, gave positive LTT responses to CMV antigen. When the same groups of patients were tested for responsiveness to HSV antigen by the LTT, there was good agreement between a positive result by this test and by serology in all except those with primary CMV mononucleosis (42.8%). Overall, lymphocyte responses to CMV were significantly impaired in healthy, CMV antibody-positive subjects from West Africa and the Middle East compared to similar subjects from Britain.

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KEY WORDS: cytomegalovirus, herpes simplex virus, lymphocyte proliferation, genitourinary infection, hemophilia, AIDS, mononucleosis

INTRODUCTION

Lymphocyte transformation tests (LTT) measure the capacity of lymphocyte preparations to undergo proliferation following exposure to antigen or mitogens and have provided useful information on cellular immune responsiveness to cytomegalovirus (CMV) infection in various groups of patients. Thus, LTT responses to CMV are depressed in congenitally infected babies and their mothers and remain low for several years especially when the infection is accompanied by disease [Gehrz et al., 1977; Reynolds et al., 1979; Starr et al., 1979]. After primary CMV infection during pregnancy, a depressed CMV lymphocyte response signifies a greater likelihood of transmission of the virus to the fetus [Stern et al., 1986; Fernando et al., 1993]. Following neonatal infection with CMV, specific lymphocyte responses may remain depressed for several years [Pass et al., 1981], probably for as long as the child continues to excrete the virus [Tanaka et al., 1986]; this is in contrast to neonatal infection with herpes simplex virus (HSV), in which significant lymphocyte responses are detected soon after diagnosis [Pass et al., 1981]. Impaired lymphoproliferative responses to CMV and other viruses (e.g., HSV) and to mitogenic lectins [Levin et al., 1979] often last for several months following primary CMV mononucleosis in adults and are associated with increased numbers of circulating $CD8^+$ suppressor cells [Carney et al., 1983]. Lymphoproliferative responses in general are depressed in transplant recipients [Quinnan and Ennis, 1980], hemophiliacs [Mahir et al., 1988], and patients infected with human immunodeficiency virus (HIV) [Lane et al., 1985].

We have reported previously that CMV IgG-positive

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Address reprint requests to Dr. J.C. Booth, Department of Medical Microbiology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, United Kingdom.

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healthy young adults from Britain and Iraq responded differently to CMV antigen in the LTT [Booth et al., 1987], in that all the British seropositives but only 51.5% of the Iraqis were positive by the LTT. Earlier, Faix et al. [1983] had noted a lower prevalence of positive CMV-LTT responses in CMV antibody-positive young women from low—compared to high—socioeconomic backgrounds in the United States. We now report the results of a further, more extensive, comparison of lymphocyte responses to CMV in adults from Britain, West Africa, and the Middle East. In agreement with previous results [Booth et al., 1987], positive CMV-LTT responses were detected in a higher proportion of CMV IgG-positive British subjects than in CMV IgG-positive subjects from West Africa and the Middle East. For the sake of further *authenticating* this finding, we have also studied groups of patients for which there is published evidence, by others, on lymphocyte responsiveness to CMV.

MATERIALS AND METHODS

Specimens and Subjects

Specimens of clotted blood and anticoagulated blood (preservative-free heparin, 10 units/ml) were collected, with agreement, from 87 Caucasians from London and elsewhere in the United Kingdom (blood donors, clerical staff, laboratory staff, and students); 60 patients attending the genitourinary medicine (GUM) clinics of St. George's Hospital and an associated hospital comprising 40 Caucasians (36 from Britain and 1 each from the United States, Italy, New Zealand, and Australia), 9 from West Africa, 6 of Afro-Caribbean origin, and 5 of Asian or Middle Eastern origins; 30 hemophiliac patients attending this hospital; 10 male homosexuals with clinical evidence of acquired immunodeficiency syndrome (AIDS); 27 patients with a glandular fever-like illness whose serum specimens had been sent to this laboratory for diagnostic investigations and had been found to be positive for both CMV IgG and CMV IgM; 37 West Africans (35 Nigerians and 2 Ghanaians), all of them students or business personnel temporarily residing in Britain; 61 blood samples from the Middle East of which 35 were from Kuwait and Egypt (students, laboratory workers, military and hospital personnel), all of which reached our laboratory within 24 hr of collection; 26 others were collected in London from Middle Eastern students, clerical and laboratory workers residing temporarily in the United Kingdom. All blood samples were collected and the tests carried out during 1981–1984.

Antigen Preparation

Monolayers of diploid human embryonic lung fibroblasts (MRC5) showing generalized cytopathology after infection with CMV strain AD169 were extracted for antigen as described previously [Booth et al., 1979]; HSV antigen (type 1, local isolate) and control antigen (from uninfected cells) were prepared in the same way. All antigen extracts were heated at 56°C for 30 min to destroy residual infectivity before use [Møller-Larsen et al., 1975/1976].

LTT

Mononuclear cells were isolated from 10 ml of heparinized blood by layering onto an equal volume of Ficoll-Paque (Pharmacia, St. Albans, Herts, UK) and spinning at 500*g* for 20 min. The banded cells were washed 3 times in RPMI 1640 medium containing 10 units/ml preservative-free heparin, then resuspended to 1×10^6 viable cells/ml in RPMI 1640 containing 15% autologous plasma. The LTT was carried out in flat-bottomed tissue culture grade microtiter plates (Sterilin, Staffs, UK: M29ARTL) seeded with 200 μl/well of this cell suspension. Aliquots (10 μl) of viral antigen (in 4-fold dilutions, starting at 1:4: [titer in the LTT = 128]), control antigen (1:4), and medium alone were, in each case, added to 4 of the wells containing the cell suspension then incubated at 37°C in 5% CO_2 in air. On the third day, 10 μl of phytohemagglutinin (PHA; 500 μg/ml in RPMI 1640; Wellcome, Beckenham, Kent, UK) was added to 4 of the culture wells that had been set up on day 0. On day 5, all of the wells received 10 μl of tritiated thymidine (Amersham TRK61, Buckinghamshire, UK, adjusted to 2 Ci/mM and 75 μg/ml total thymidine). The cells were harvested onto glass fiber (Whatman GF/C, Maidstone, Kent, UK) filter disks 24 hr later and counted in an LKB1215 RackBetaII liquid scintillation counter (Wallac, Milton Keynes, Bucks, UK). The results of the test were expressed as stimulation index (SI) values which were calculated as the mean cpm for the virus-stimulated cultures divided by the mean cpm for the cultures with the control antigen or, in the case of the cultures treated with PHA, by the mean cpm for the cultures inoculated with medium in place of antigen.

Serological Testing

Tests for CMV IgG, CMV IgM, and HSV IgG were by indirect enzyme immunoassay (EIA) using standard laboratory procedures [Booth et al., 1979; Kangro et al., 1984] with the same antigens as in the LTT; quantitative differences between groups of sera were assessed on the basis of the EIA absorbance reading that was recorded on testing the sera at a dilution of 1:300. Testing for antibody to HIV was by the Abbott 1 + 2 test (Abbott Laboratories, North Chicago, IL).

RESULTS

Optimization of the LTT Response to CMV Antigen

As shown previously [Booth et al., 1987], LTT responses to CMV antigen in CMV antibody-positive subjects were higher when CMV antibody-positive plasma, compared to CMV antibody-negative plasma, was present in the culture medium. Moreover, when CMV antibody-positive donors were tested against CMV antigen in

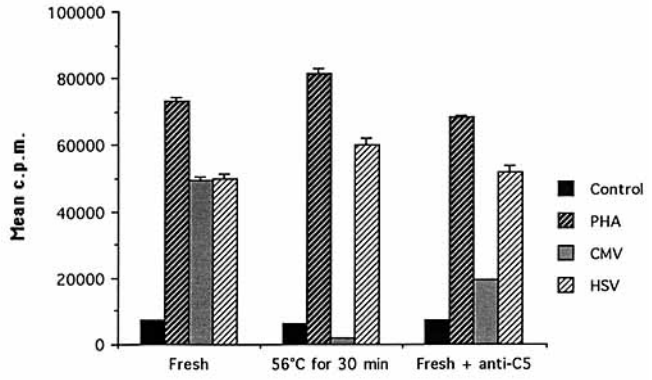

Fig. 1. Effect of unheated autologous plasma, heated (56°C for 30 min) autologous plasma, and unheated autologous plasma with added anti-human C5 antibody on the lymphocyte proliferation responses to CMV antigen, HSV antigen, control antigen, and PHA in a healthy adult Caucasian subject antibody-positive to CMV and HSV.

the LTT, the SI values were higher with fresh autologous plasma than with heated (56°C for 30 min) autologous plasma in the culture medium (Fig. 1). On the other hand, the use of heated autologous plasma had no significant effect on the LTT responses to HSV antigen in HSV antibody-positive subjects, nor on the responses to PHA.

Depletion of complement by adding 10 μl of rabbit anti-human C5 immediately after setting up the LTT cultures in the presence of fresh autologous plasma also significantly reduced the SI responses to the CMV antigen, although not as severely as when heated autologous plasma alone was used (Fig. 1); the responses to HSV and PHA were unaffected by the addition of the anti-C5.

As a result of these experiments, the LTT was put up routinely using medium containing unheated autologous plasma.

Specificity of the LTT

This was shown in the course of testing a CMV antibody-negative patient at intervals before and after a subcutaneous injection of CMV live vaccine (strain AD169; 10,000 TCD50 [Elek and Stern, 1974]), which was given prior to the patient's receiving a kidney transplant from a CMV antibody-positive donor (Fig. 2). Before vaccination, the LTT responses to the CMV antigen were clearly negative and they remained so until 3 weeks after vaccination when a significant proliferative response was detected which persisted, with some fluctuation, beyond week 42. The highest overall SI value was recorded in the 4th week after vaccination. Consistent with the patient's being positive for HSV IgG at the outset, positive LTT responses to this virus were obtained at each time of testing, and significant responses to PHA (these results are not shown in Fig. 2). CMV IgG was first detected, by EIA, at week 3 post-vaccination. Similar tests were also carried out on a second vaccinee who, before vaccination, was negative for antibody to HSV as well as to CMV: positive LTT responses to CMV were detected first at week 2 post-vaccination and in 6

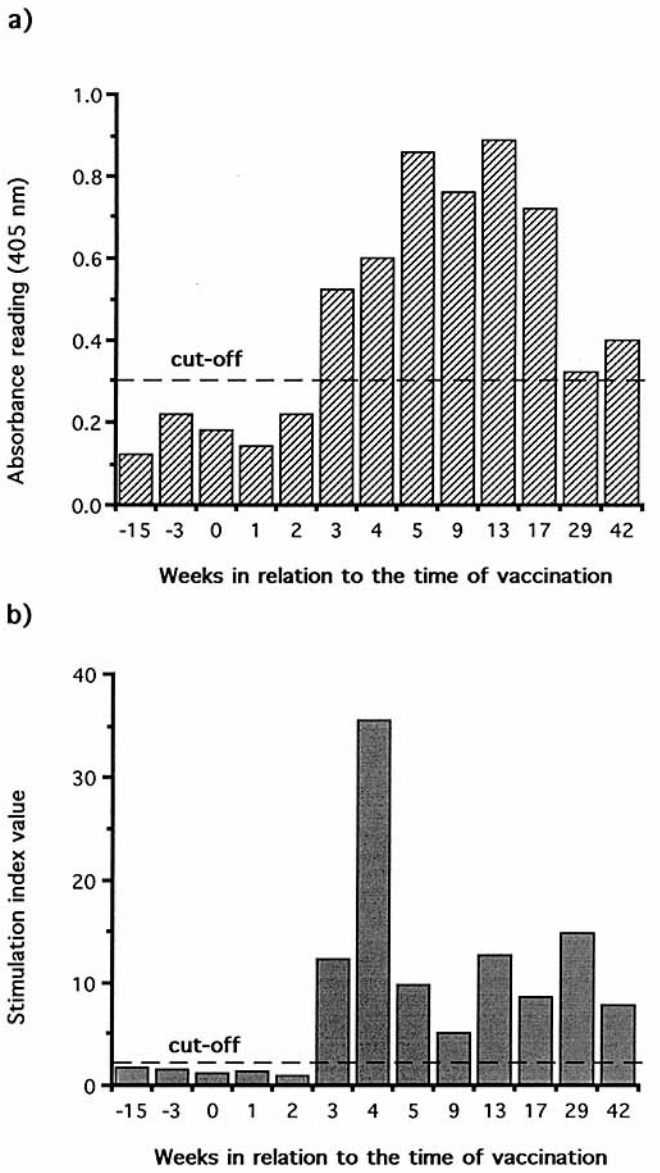

Fig. 2. Immune response to live CMV vaccine in a CMV antibody-negative patient given a single dose of live CMV vaccine prior to a kidney transplant from a CMV antibody-positive donor. **a:** EIA tests for CMV IgG. **b:** LTT with CMV antigen.

further specimens collected up to week 29; CMV IgG was detected after week 9; HSV LTT responses were consistently negative up to week 29 (results not shown).

Reproducibility of Responses by the LTT

Two healthy adults who were positive for antibodies to both CMV and HSV were tested by the LTT at intervals of 1–7 months over 2 years. The coefficients of variation for the SI values were, respectively, 32% and 35% against the CMV antigen, 22% and 27% against the HSV antigen, and 34% and 38% against PHA.

TABLE I. Prevalence of Antibody to CMV and HSV in Lymphocyte Donors

	No. antibody-positive/ No. tested	
	CMV	HSV
British		
Healthy controls	44/87 (50.5%)	52/84 (61.9%)
GUM patients	46/60* (76.6%)	43/57 (75.4%)
Hemophiliacs	21/30* (70%)	15/29 (51.7%)
AIDS patients	10/10* (100%)	10/10* (100%)
CMV mononucleosis patients	27/27* (100%)	10/18 (55.5%)
West African		
Healthy controls	33/37* (89.1%)	35/37* (94.5%)
Middle Eastern		
Healthy controls	54/61* (88.5%)	34/40* (85%)

*$P < 0.05$ relative to the British healthy control group according to the chi-squared 2×2 test.

TABLE II. Comparison of Antibody Levels to CMV and HSV in Lymphocyte Donors as Determined by Indirect EIA

	Mean antibody level (SD)	
	CMV	HSV
British		
Healthy controls	0.71 (0.17)	0.72 (0.17)
GUM patients	0.94* (0.21)	1.01* (0.32)
Hemophiliacs	0.76 (0.35)	0.63 (0.15)
AIDS patients	0.98* (0.34)	0.99* (0.23)
CMV mononucleosis patients	1.12* (0.39)	1.01* (0.27)
West African		
Healthy controls	0.68 (0.32)	0.88* (0.19)
Middle Eastern		
Healthy controls	0.80* (0.23)	0.76 (0.25)

*$P < 0.05$ relative to the British healthy control group according to the Student's unpaired t-test.

Ages of the British, West African, and Middle Eastern Subjects and the Patients

The mean ages for the GUM, the AIDS, and the hemophiliac patients were significantly lower ($P < 0.05$) than for the British control subjects (30.5, 28, and 35.6 years, respectively); the data for the CMV mononucleosis patients were incomplete so that a reliable comparison could not be made. The mean ages for the West African and the Middle Eastern control subjects (31 and 31.8 years, respectively) were not significantly different ($P > 0.05$) from those for the British control subjects.

Serological Testing of the Control Subjects and the Patients

Compared with the British control subjects, significantly more of the West African and the Middle Eastern control subjects, and of each of the various groups of patients, were positive for CMV IgG (Table I). When tested for HSV IgG, however, only the AIDS patients and the West African and the Middle Eastern control subjects showed a higher prevalence than the British controls (Table I). CMV IgM antibody was detected only in the patients with CMV mononucleosis.

The mean IgG antibody levels against CMV and HSV were significantly higher in the GUM, AIDS, and CMV mononucleosis patients, but not in the hemophiliacs, when compared to the British controls (Table II). In the West African control subjects, the mean level of CMV IgG was not significantly different from that for the British controls, but the mean level of HSV IgG was significantly higher. In the Middle Eastern group, the mean CMV IgG level, but not the level for HSV IgG, was significantly higher than in the British controls (Table II).

LTT on the British Control Subjects and on the Patients

British control subjects. In the CMV LTT, all 44 who were CMV IgG-positive gave SI values of greater than 2.1, whereas the 43 who were CMV IgG-negative gave SI values of less than 1.9; an SI value of equal to or greater than 2.1 was therefore regarded as the cutoff for distinguishing between positive and negative responses in the LTT (Table III). A positive result, based on this criterion, was confirmed if the mean cpm with the virus antigen was significantly different ($P < 0.05$) from the mean cpm with the control antigen by the same LTT, according to the Student's t-test. The median SI value for all those who were CMV IgG-positive was 4.7 (Table IV).

Fifty of the subjects were tested by LTT against the HSV antigen. The SI values ranged from 0.3 to 34.2 for the 36 who were HSV IgG-positive and 0.5 to 1.4 for the 14 who were HSV IgG-negative. Two of the antibody-positives gave SI values of 0.3 and 1.1, but the next highest value was 2.1; hence, by regarding SI ≥ 2.1 as the cutoff, and by applying the same confirmatory t-test calculation as for CMV, 34 (94.4%) of the 36 HSV IgG-positives were judged to be positive by the HSV LTT (Table III). The median SI value for the HSV antibody-positives was 5.7 (Table IV).

GUM patients. Of the 46 patients who were positive for CMV IgG, significantly fewer (86.9%) were positive against the CMV antigen by the LTT than in the group of CMV IgG-positive healthy control subjects (Table III); the median SI values were not significantly different in both (Table IV). Three of the discordant results were in patients from West Africa (Nigeria 1, Ghana 2).

TABLE III. LTT Against CMV and HSV in Antibody-Positive Lymphocyte Donors

	No. positive by LTT/ No. tested (%)	
	CMV	HSV
British		
Healthy controls	44/44 (100%)	34/36 (94.4%)
GUM patients	40/46* (86.9%)	43/43 (100%)
Hemophiliacs	14/21* (66.6%)	13/13 (100%)
AIDS patients	2/10* (20%)	3/4 (75%)
CMV mononucleosis patients	13/27* (48.1%)	3/7* (42.8%)
West African		
Healthy controls	25/33* (75.7%)	28/28 (100%)
Middle Eastern		
Healthy controls	30/54% (55.5%)	34/34 (100%)

*$P < 0.05$ relative to the British healthy control group according to the chi-squared 2 × 2 test.

On the basis of declared lifestyle, 4 of those who were CMV antibody-positive were judged to be at risk for infection with HIV; permission for testing for antibody to HIV could not be obtained but 3 gave negative responses by the CMV LTT.

Forty-three patients were positive for HSV IgG and against the HSV antigen by the LTT (Table III) and gave a median SI value that was significantly greater than in the HSV antibody-positive control subjects (Table IV). Three of the 4 patients at risk for acquiring HIV were positive both for HSV IgG and by the HSV LTT; the fourth patient was negative by both of these tests.

All but one of the GUM patients who were negative for CMV IgG were also negative by the LTT; the discrepancy gave an SI value of 5.9 in an Asian patient. All who were negative for HSV IgG were negative against this virus by the LTT.

In the other groups of patients who were examined in this study (see below), and in the West African and Middle Eastern control subjects, there was complete agreement between a negative serological result for CMV or HSV and a negative SI value by the corresponding LTT.

Hemophiliacs. Of the 21 patients who were positive for CMV IgG, significantly fewer (66.6%) than in the healthy British control subjects were positive against the CMV antigen by the LTT (Table III); the median SI value against CMV was also significantly lower than for the control subjects (Table IV).

Twenty-nine of the patients had been tested for antibody to HIV as part of their clinical follow-up; 14 were positive including 10 who were also positive for CMV IgG; among these 10 were 4 of the 7 patients who tested negative against the CMV antigen by the LTT. On dividing the CMV antibody-positive patients according to their HIV status it was found that 6 of the 10 HIV-positive patients were positive by the LTT against CMV and 8 of the 11 who were negative for antibody to HIV; in both cases the proportions were significantly different from the U.K. controls ($P < 0.001$).

Thirteen hemophiliacs positive for HSV IgG were also positive against HSV by the LTT (Table III) and gave a median SI value not significantly different from that for the British control group positive for HSV IgG (Table IV); 4 of the 13 (30.7%) were positive for antibody to HIV.

Patients with clinical AIDS. All 10 patients were positive for antibody to HIV, CMV, and HSV. Only 2 gave positive responses against CMV by the LTT (Table III), which was significantly fewer than in the control group; the median SI against the CMV antigen was significantly lower than in the controls (Table IV). Three (75%) of the 4 patients who were tested by the LTT against the HSV antigen gave positive results (Table III), which was not significantly different from the control subjects; the median SI value for these 4 patients was significantly lower than for the healthy controls (Table IV).

CMV mononucleosis patients. All 27 patients had presented with a glandular fever-like illness during the previous 4–14 weeks; 13 (48.1%) were positive against CMV by the LTT (significantly fewer than in the control group; Table III) with a median SI value significantly lower than in the control group (Table IV). Insufficient blood was available for testing against HSV antigen by the LTT.

The median SI value against PHA was significantly lower, compared with the control group, in the hemophiliac and CMV mononucleosis groups but not in the GUM group nor the patients with AIDS (Table IV).

West African and Middle Eastern control subjects. Compared with the British controls, significantly fewer of those who were positive for CMV IgG were positive against the CMV antigen by the LTT (75.7% of the West Africans and 55.5% of those from the Middle East; Table III); the median SI values were significantly lower in both groups than in the British controls (Table IV).

Against the HSV antigen, all those in both groups who were antibody-positive were also positive by the LTT (not significantly different from the British control group; Table III); the median SI values against HSV and PHA were not significantly different from those in the British control group (Table IV).

DISCUSSION

The finding that CMV antibody-positive healthy subjects from West Africa and the Middle East are less able to mount a detectable LTT response to CMV when compared with healthy British subjects complements a previous study from this laboratory on adults native to Britain and Iraq [Booth et al., 1987]. The results of testing the British control subjects and the various groups of patients are in general agreement with the experience of others, which suggests that the observed differences between the various ethnic groups were not due to technical reasons. Thus, the proportion of CMV

TABLE IV. SI Values in LTT Against CMV, HSV, and PHA

	Median SI values (range) against		
	CMV	HSV	PHA
British			
Healthy controls	4.7 (2.1–24.8)	5.7 (1.1–41.9)	24.7 (4.8–170.1)
GUM patients	6.7 (0.3–44.1)	6.7* (2.1–58.0)	23.4 (5.5–126.8)
Hemophiliacs	3.2* (0.6–43.4)	5.2 (2.1–14.1)	19.1 (9.6–117.9)
AIDS patients	1.4* (0.9–12.7)	2.2* (1.0–15.3)	15.4 (3.7–143.6)
CMV mononucleosis patients	2.0* (0.3–15.8)	ND	14.0* (0.9–93.6)
West African			
Healthy controls	3.0* (1.1–70.5)	6.0 (2.0–31.7)	27.0 (5.4–96.3)
Middle Eastern			
Healthy controls	2.4* (0.2–25.7)	5.7 (1.9–15.8)	22.6 (10.2–97.4)

*$P < 0.05$ relative to the median value for the British healthy control group according to the Mann-Whitney U-test.

antibody-positive control subjects (50.5%) is in keeping with published information for the population of London [Stern and Elek, 1985]. The higher prevalence of CMV antibody in the GUM patients (76.6%) may reflect a lifestyle predisposing to increased risk of CMV infection and, in the case of the hemophiliacs (70%), past exposure to blood; otherwise, being significantly younger, the prevalence of CMV antibody in these two groups of patients ought to have been lower than in the U.K. control subjects. The high prevalence (100%) of CMV antibody in the AIDS patients was as expected [Schooley, 1990] and is, of course, to be expected for recent CMV mononucleosis.

The prevalence of antibody to HSV in the British control group (61.9%) agrees with the findings of others [Ades et al., 1989]. Our in-house EIA did not distinguish between antibodies specific for HSV types 1 and 2, which probably explains why there were no significant differences in prevalence between the control subjects and the GUM patients (75.4%), the hemophiliacs (51.7%), and the CMV mononucleosis patients (55.5%). The higher prevalence of HSV antibody in the AIDS patients and in the West African and the Middle Eastern subjects reflects well-known epidemiological patterns [Nahmias et al., 1989].

Our procedure for the LTT has been applied in several studies [Booth et al., 1987; Fernando et al., 1993; Stern et al., 1986]. Fresh autologous plasma in the lymphocyte culture medium ensures the presence of CMV antibody which is known to potentiate the specific stimulation of lymphocytes from CMV antibody-positive individuals [Schirm et al., 1983; Booth et al., 1987]; it is also a source of complement which, in our hands, also enhances lymphocyte responses to CMV, as has been shown in other systems [Sundsmo, 1983; Morgan et al., 1983]. Neither specific antibody nor complement is as important for maximizing lymphoproliferative responses to HSV. Perhaps this is because, CMV being a lymphotropic virus, the uptake of antigen by antigen-presenting cells may be more readily increased by humoral factors than in the case of HSV.

The specificity of the LTT procedure was demonstrated in the example of the renal transplant recipient given live CMV vaccine. The first detection of CMV LTT activity and specific antibody occurred at times consistent with the work of others [Gehrz et al., 1980; Starr et al., 1981].

In the tests with the HSV antigen, there was good agreement between serological status and a positive or negative LTT result in both the GUM patients and the hemophiliacs as well as in the British control group. In the patients with AIDS and those with CMV mononucleosis, the association was less complete, albeit proliferative responses to a wide range of antigens are impaired in such patients [Levin et al., 1979; Janossy et al., 1993; Lane et al., 1985].

In the tests with the CMV antigen, the only complete correlation between serological status and LTT response was in the British control subjects. In the groups of GUM patients, hemophiliacs, AIDS patients, and mononucleosis patients, 13–80% of those who were CMV antibody-positive were negative by the LTT. Such discordance is not unexpected in AIDS patients, nor in patients with recent CMV mononucleosis. Indeed, in the latter, proliferative responses to CMV and HSV, and to mitogenic lectins, are known to remain depressed for several weeks after the acute infection [Levin et al., 1979; Ten Napel and The, 1980]. In hemophiliac patients, proliferative responses to antigens and PHA are depressed because of repeated exposure to allogeneic proteins in clotting factor concentrates [Wang et al., 1985]. In some of the hemophiliacs in the present study who gave false negative responses to CMV by the LTT, this may have been because they were infected with HIV. In the GUM patients who were positive for antibody to CMV, the CMV LTT responses were negative in some who were known

to have risk factors for HIV, although this could not be substantiated by serological testing because of consent being withheld.

The high proportion of discordant results, in which those who were CMV antibody-positive were found to be negative by the LTT, among the West African and Middle Eastern subjects (24.3% and 44.5%, respectively), argues against these discordant results being due mainly to infection with HIV. Such high prevalences of HIV infection would be exceptional in unselected healthy adults and would have been especially so at the time the study was carried out, i.e., 1981–1984. Testing these subjects for HIV antibody was precluded because of the difficulty of obtaining consent.

Significant LTT responses to HSV antigen were detected in most of the CMV antibody-positive subjects who gave negative or weak responses by the LTT with CMV. This indicates that non-specific inhibitors of lymphocyte proliferation were absent from the specimens under test and that the patients' mononuclear cells were intrinsically fully capable of presenting and recognizing viral antigens and of elaborating cytokines. The false negative or weak LTT responses to CMV antigen, in particular, might have been because those patient's lymphocytes that were predisposed to respond to CMV were already maximally expanded at the time of testing, because of repeated stimulation in vivo following either frequent reactivation of latent endogenous CMV or repeated exogenous reinfection. The prevalence of CMV reactivation in normal healthy adults is known from studies on seropositive pregnant women in Europe and North America, and is in the region of 3–5% [cited in Stern, 1977]. The detection of significant LTT responses to HSV in the face of false negative responses to CMV also argues against a generalized immunosuppression due to intercurrent infection with other microbial agents.

Another possibility was that those CMV antibody-positive patients who responded poorly by the LTT had suffered deletion of CMV-specific T cell clones following intrauterine or perinatal infection, presupposing a failure of such cells to be effectively reconstituted with time. Long-term tolerance to CMV could arise because of persistence of the virus in the thymus or other lymphoid tissue, as has been shown for lymphocytic choriomeningitis virus in mice [Tishon et al., 1993]. Alternatively, non-Caucasians may be predisposed, more so than Caucasians, to generate CMV-specific suppressor T cells, or the AD169 strain of CMV, which was used for preparing the antigen in this study, might be more akin to wild-type strains circulating in Britain rather than in West Africa and the Middle East. Levels of CMV IgG in the West African and Middle Eastern subjects were either no different from those in the British control subjects or significantly higher, which argues against a severe functional defect in Th_2 cell responses [Milich et al., 1995].

We have speculated previously that differences in lymphocyte responsiveness to CMV in adults may be linked to the age at which primary infection occurs [Booth et al., 1987]. The selectivity of the lymphoproliferative defect for CMV, rather than HSV, resembles the findings of Pass et al. [1981] that, whereas neonates who became infected with HSV mounted significant LTT responses to that virus, neonates who became infected with CMV did not develop detectable CMV LTT reactivity for several years. In Middle Eastern and West African societies CMV is usually acquired in the first year of life, whereas in Britain it occurs mostly during childhood, adolescence, and later [Stern and Elek, 1965]. Thus, to a greater or lesser extent, impaired lymphoproliferative responsiveness following CMV infection in early life might be prolonged into adult life; this could explain the higher prevalence of congenital CMV infection in West African [Bello and Whittle, 1991] than in Western European societies. In West Africa, most women already have long-standing latent or persistent CMV infection by the time they become pregnant, so that most cases of intrauterine infection in such populations are the consequence of either reactivation of latent CMV in the mother or exogenous reinfection, the likelihood of which would be enhanced by diminished cell-mediated immune responsiveness to CMV.

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